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Effects of Cigarette Smoking on Metabolic Events in the Lung

by Satoshi Kitamura*

Nicotine and cigarette smoke extract show acute physiological effects: increasing tracheal pressure (P_{TR}), pulmonary artery pressure (P_{PA}), systemic blood pressure (P_{SYST}), and left atrium pressure (P_{LA}); and decreasing cardiac output ($\dot{Q}_{AORTA}$) and blood flow to the left lower lobe ($\dot{Q}_{LLL}$). In addition, cigarette smoking induces bronchoconstriction, thus decreasing peak flow, FVC, and $FEV_{1.0}$ in healthy subjects. It has also been demonstrated that cigarette smoking caused temporary slowing of mucociliary clearance in the lung and that cigarette smoke increases the activity of aryl hydrocarbon hydroxylase which metabolizes benzo[α]pyrene. We demonstrated that serum angiotensin I converting enzyme (ACE) activity showed a significant increase immediately after smoking and returned to the control level 20 min after smoking. We also demonstrated that plasma histamine levels showed a marked decrease after smoking. Furthermore, the effects of cigarette smoke and related substances on prostaglandin, thromboxane, testosterone, cyclic nucleotides metabolism, and protein synthesis were also investigated.

Introduction

Cigarette smoke contains hundreds of gaseous and particulate constituents, many of which are cytotoxins (*1*). The adverse physiological and biochemical effects exerted by cigarette smoke on an active smoker have been alluded to, if not clearly demonstrated. Cigarette smoking is an important risk factor in lung cancer, chronic obstructive lung disease, and coronary heart disease (*2,3*).

The fundamental importance of the lung in providing oxygen and eliminating carbon dioxide is well known. However, the lung has an another important role—the metabolism of vasoactive substances (*4*). In this brief review, the effects of cigarette smoking on various metabolic events in the lung *in vitro* and *in vivo* are presented.

Cardiopulmonary Effects of Tobacco and Related Substances

Effects of Nicotine and Cigarette Smoke Extract Injection on the Canine Airway and Pulmonary and Systemic Circulation (*5*)

It is well known that cigarette smoking has diverse effects not only on the airway system but also on the pulmonary and systemic circulatory system. The acute effects of cigarette smoking are induced mainly by nicotine, the main alkaloid in tobacco. This investigation was conducted to explore the acute effects of nicotine and cigarette smoke extract on canine airway, pulmonary, and systemic circulatory systems *in vivo*. Twelve dogs, weighing between 18 and 25 kg, were anesthetized with intravenous adminstration of 25 mg/kg of sodium pentobarbital. The trachea of each dog was cannulated for artificial ventilation with a volume-type Harvard respirator, and the left femoral artery and vein were catheterized for measurement of systemic blood pressure (P_{SYST}), and for infusing drugs such as heparin Na (100 U/kg) or succinyl choline chloride (2%, 0.2 mL/kg/hr). The left hemithorax was opened under artificial respiration, and catheters were inserted into the pulmonary artery trunk and left atrium for measurement of pulmonary artery pressure (P_{PA}) and left atrium pressure (P_{LA}). The noncannulating electromagnetic flow probes were placed around the ascending aorta and pulmonary artery of the left lower lobe for measurement of cardiac output ($\dot{Q}_{AORTA}$) and blood flow to the left lower lobe ($\dot{Q}_{LLL}$), respectively. Tracheal pressure (P_{TR}) was measured at the orifice of the tracheal cannula using a pressure transducer. Nicotine and cigarette-smoke extract were injected into the left pulmonary artery through a polyethylene catheter using a Harvard low speed infusion pump. P_{TR}, P_{PA}, P_{LA}, P_{SYST}, $\dot{Q}_{AORTA}$, and $\dot{Q}_{LLL}$ were recorded and displayed on an electric polyrecorder (Nihon Kohden, Japan) through an amplifier. Figure 1 illustrates the experimental arrangement. The saline solution of cigarette smoke extract was prepared by smoking a cigarette (Hilite, one of the popular cigarettes manufactured by the Japan Monopoly Corporation) through an aqua-filter containing 2 mL of water and diluted with 20 mL of saline.

Figure 2 is a typical record of various parameters changed by an injection of nicotine solution into the pul-

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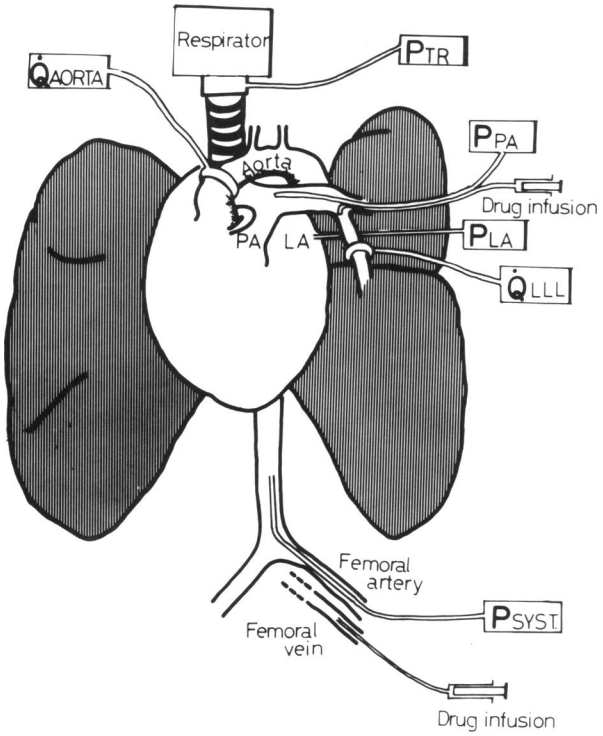

FIGURE 1. Experimental arrangement showing positioning of probes for the measurement of pulmonary artery pressure (P_{PA}), left atrium pressure (P_{LA}), systemic blood pressure (P_{SYST}), cardiac output ($\dot{Q}_{AORTA}$), tracheal pressure (P_{TR}), and blood flow to left lower lobe ($\dot{Q}_{LLL}$). Drugs were infused through the femoral vein and left pulmonary artery as indicated.

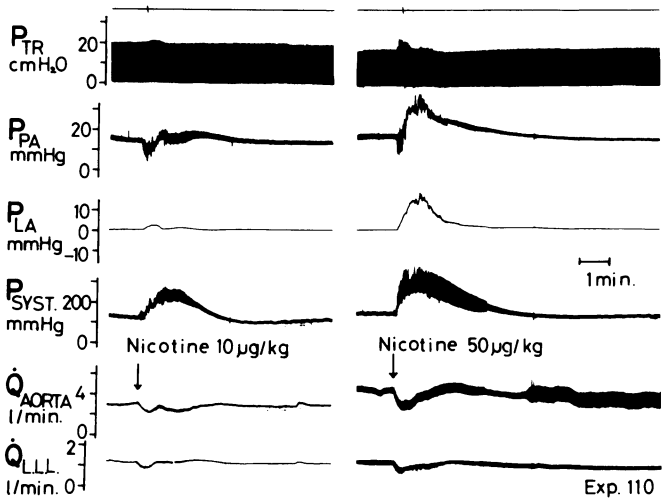

FIGURE 2. A typical record of various parameters changed by an injection of nicotine solution into the pulmonary artery of the left lower lobe in an anesthetized dog.

monary artery of the left lower lobe in an anesthetized dog. Injection of nicotine (50 μg/kg) induced a transient increase of P_{TR}; a sharp increase of P_{PA} following an initial slight decrease; an increase of P_{LA}; a marked increase of P_{SYST}; and a decrease of $\dot{Q}_{AORTA}$ and $\dot{Q}_{LLL}$ followed by slight increases. The lower dose (10 μg/kg) of nicotine showed almost the same tendency. Figure 3 shows changes of P_{PA}, P_{LA}, and P_{SYST} by injection of various doses of nicotine into the pulmonary artery of the left lower lobe. It is obvious from this figure that P_{PA}, P_{LA}, and P_{SYST} showed a marked increase in their dose-dependence.

Figure 4 is the summary of experimental data from eight dogs, showing changes in P_{TR}, P_{PA}, and P_{SYST} with increasing doses of nicotine. P_{PA} and P_{SYST} showed significant increases in their dose-dependence, while P_{TR} tended to increase but without significant differences (Student's unpaired *t*- test).

Figure 5 is the summary of experimental data from eight dogs showing changes in P_{LA}, $\dot{Q}_{AORTA}$, and $\dot{Q}_{LLL}$ with increasing doses of nicotine. P_{LA} showed a significant increased dose-dependence, while $\dot{Q}_{AORTA}$ and $\dot{Q}_{LLL}$ showed significant decreases in their dose-dependence.

Figure 6 is a typical record of various parameters changed by an injection of 2 mL of saline solution of cigarette smoke extract into the pulmonary artery of the left lower lobe in an anesthetized dog. Injection of cigarette smoke extract solution elicited a slight increase of P_{PR}; an increase of P_{PA} following an initial slight decrease; a marked increase of P_{SYST}; and decreases of $\dot{Q}_{AORTA}$ and $\dot{Q}_{LLL}$ followed by increases. The results suggest that nicotine and cigarette smoke extract show acute physiological effects increasing P_{TR}, P_{PA}, and P_{SYST}, and decreasing $\dot{Q}_{AORTA}$ and $\dot{Q}_{LLL}$.

Effects of Cigarette Smoking on Pulmonary Functions in Healthy Volunteers (*6*)

Twenty-three healthy nonasthmatic volunteers (20 men and 3 women) were studied. They ranged in age from 22 to 47 years, averaging 33.3 years. All of them were heavy smokers (20–40 cigarettes/day) for 5 to 20 years. The volunteers stopped smoking at least 12 hr before testing began. When testing began, men smoked five cigarettes, and women smoked three cigarettes within 10 min. Men smoked more cigarettes than women to account for the lower body weight of the women (10–15 kg less than men). Several of the volunteers showed symptoms such as dizziness or palpitation. Peak flow, forced vital capacity (FVC), and one-second forced expiratory volume (FEV1.0) were measured before, immediately after, and 20 min after smoking. Cigarettes used in this experiment were Hilite, made by Japan Monopoly Corporation.

Figure 7 shows the effect of cigarette smoking on peak flow. Mean values and standard deviations of peak flow before, immediately after, and 20 min after cigarette smoking were 568.05 ± 88.62 L/min, 543.13 ± 88.04 L/min, and 569.06 ± 93.55 L/min, respectively. Figure 8 shows the effect of cigarette smoking on FVC. Mean values and standard deviations of FVC before, immediately after, and 20 min after cigarette smoking were 4233.16 ± 658.03 mL, 3964.21 ± 65.00 mL, and 4041.05 ± 688.18 mL, respectively.

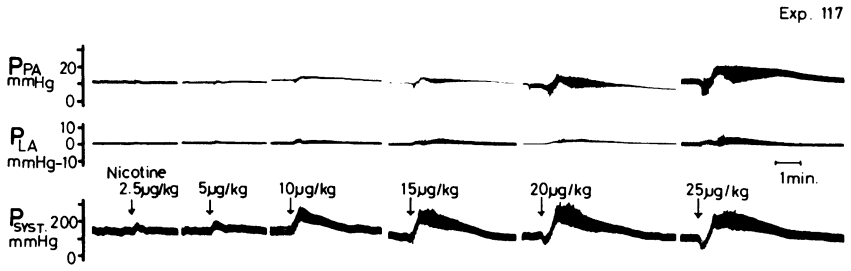

FIGURE 3. Changes of P_{PA}, P_{LA}, and P_{SYST} by injections of nicotine into the pulmonary artery of the left lower lobe.

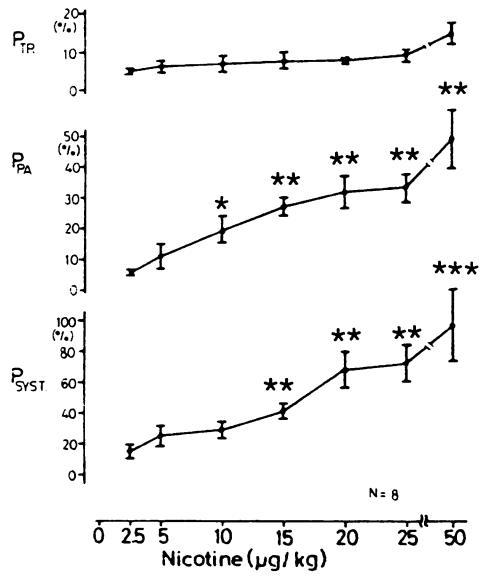

FIGURE 4. Percentage change of P_{TR}, P_{PA}, and P_{SYST} by injections of nicotine into the pulmonary artery of the left lower lobe. * $p <$ 20.02, ** $p < 0.01$, *** $p < 0.001$.

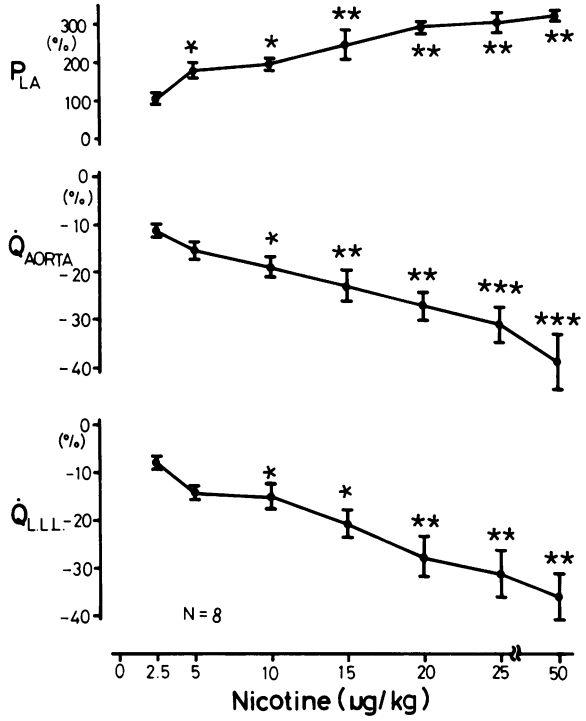

FIGURE 5. Percentage change of P_{LA}, $\dot{Q}_{AORTA}$, and $\dot{Q}_{LLL}$ by injections of nicotine into the pulmonary artery of the left lower lobe. * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$.

Figure 9 shows the effect of cigarette smoking on FEV1.0. Mean values and standard deviations of FEV1.0 before, immediately after, and 20 min after cigarette smoking were 3734.74 ± 658.60 mL, 3515.26 ± 683.82 mL, and 3476.84 ± 556.8 mL, respectively.

These results suggest that cigarette smoking induces bronchoconstriction, thus decreasing peak flow, FVC, and FEV1.0. Da Silva et al. (*7*) studied the effect of smoking one cigarette on lung function in 21 healthy subjects. Airway resistance, measured by body plethysmography, increased in 19 patients. Closing volume was not significantly changed after smoking one cigarette, although the maximum expiratory flow at 50% of the vital capacity (MEF_{50}) decreased significantly. These findings indicate that smoking one cigarette dose not increase small airway resistance measurably when procedures not requiring maximum expiratory flow are used. On the other hand, the decrease of MEF_{50} indicates that airways upstream from the flow-limiting segment have increased resistance after smoking one cigarette, since no changes in the elastic properties of the lung were demonstrated.

Effect of Cigarette Smoke on Mucociliary Clearance and on Phagocyte Function

The ciliastatic effect of cigarette smoke and smoke components has been demonstrated by direct observation of the tracheal mucosa in a variety of animals including the cow, rabbit (*8*), and cat (*9*). Mucociliary transport rates have been measured in the human nose, but few data on the effects of cigarette smoke have been reported (*10*). Frances et al. (*11*) studied the effects of whole cigarette smoke on the transport of particles on the nasociliary mucosa of the donkey. They concluded that the nasociliary mucosa was much more resistant to cigarette smoke than the tracheobronchial tree, since previous experiments on donkeys (*12*) demonstrated that exposure to the smoke from 20 cigarettes produced a marked retardation in par-

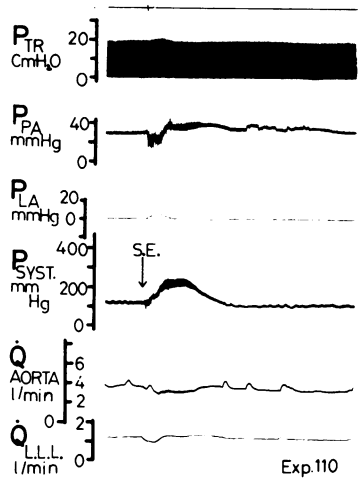

FIGURE 6. A typical record of various parameters changed by an injection of saline solution of cigarette smoke extract into the pulmonary artery of the left lower lobe in an anesthetized dog.

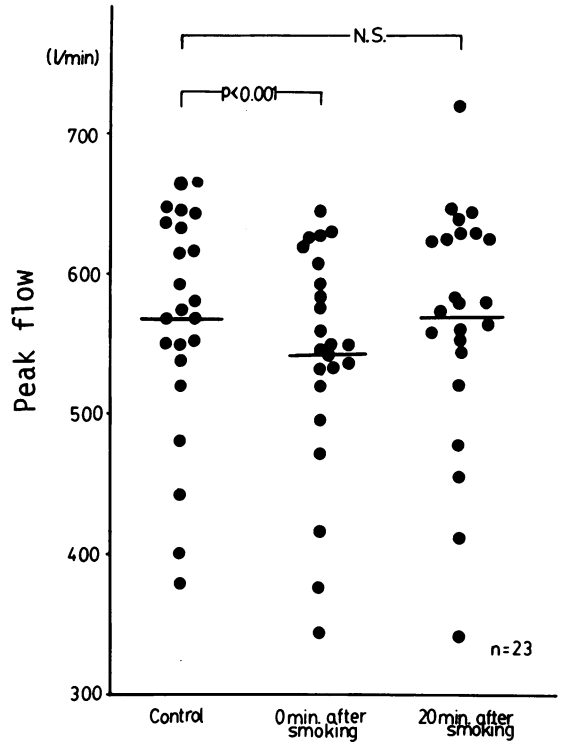

FIGURE 7. Effect of cigarette smoking on peak flow. Control values were measured before cigarette smoking. 0 min measurements were taken immediately after cigarette smoking.

ticle transport in the trachea. Pavia et al. (*13*) demonstrated that tobacco smoking caused temporary slowing of mucociliary clearance in the lung. In brief, clearance was assessed by monitoring, externally to the chest, the removal of 5 μm particles labeled with technetium-99m which had been inhaled in controlled conditions in 22 volunteers (aged 64–92; mean 74.6 years) from homes for the elderly.

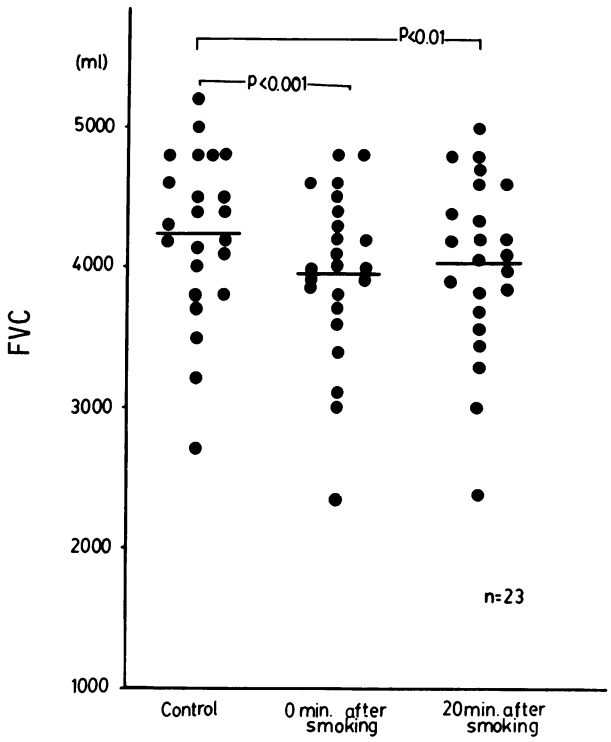

FIGURE 8. Effect of cigarette smoking on FVC.

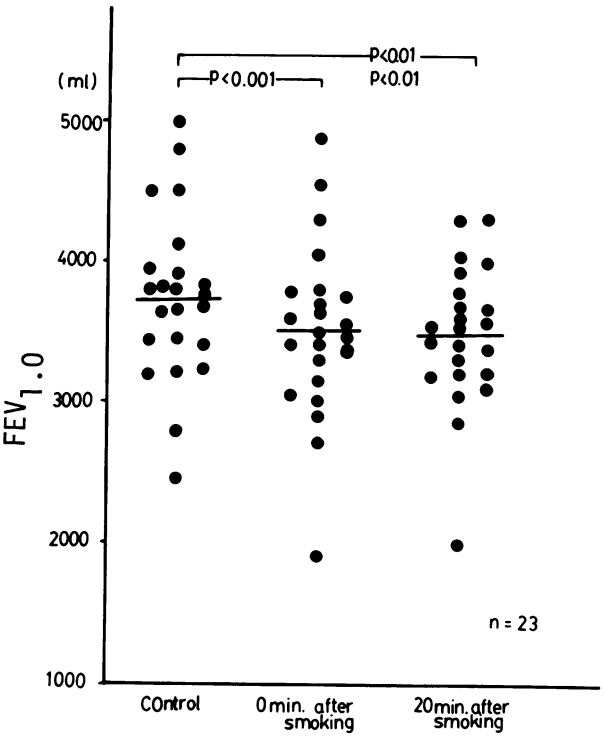

FIGURE 9. Effect of cigarette smoking on FEV1.0.

Acute exposure to cigarette smoke has been shown to inhibit the bactericidal activity of alveolar macrophages cultured *in vitro* (*14,15*) and the ability of mouse alveolar macrophages to phagocytose inhaled particles (*16*). However, the effect of chronic inhalation of cigarette smoke on bactericidal and phagocytic activities has not been elucidated yet. When investigations were conducted using alveolar macrophages recovered from cigarette smokers and nonsmokers by bronchoalveolar lavage (*17–19*), it was found that cigarette smokers had a higher number of alveolar macrophages and that their macrophages generally had a normal level of phagocytic activity.

Warr et al. (*20*) demonstrated that pulmonary alveolar macrophages from six cigarette smokers showed higher random migration and greater chemotactic responsiveness to casein than did macrophages from seven nonsmokers and suggested that pulmonary macrophages were metabolically activated by cigarette smoking. This might indicate that cigarette smokers, because of their elevated levels of alveolar macrophages, possess a more effective pulmonary phagocytic ability than nonsmokers. Rylander (*21,22*) found that guinea pigs exposed to cigarette smoke for 4 weeks developed an increased number of alveolar macrophages and pulmonary polymorphonuclear leukocytes, but the bactericidal activity of these cells measured *in situ* was markedly depressed. Because of the relatively high number of pulmonary polymorphonuclear leukocytes in guinea pigs and the relatively short period of cigarette smoke exposure used for these investigations, it is suggested that these experimental conditions might differ from the situation in the lungs of cigarette smokers.

Demarest et al. (*23*) investigated the effects of acute smoke inhalation on the random and chemotactic migration of human pulmonary alveolar macrophages (AM) obtained by fiberoptic subsegmental pulmonary lavage in 19 nonsmokers and 7 smokers. He demonstrated the *in vitro* exposure of pulmonary AM to nontoxic doses of smoke produced the impairment of AM chemotaxis and suggested that these findings may partially explain the enhanced susceptibility of smokers to pulmonary infection. The nylon fiber adherence *in vitro* of AM from cigarette smokers was uniformly decreased. Rasp et al. (*24*) demonstrated that there is a reversible, intrinsic defect in the structure and adherence of AM from cigarette smokers that may influence their function and may account, in part, for the increased yield of AM from the lavage fluid of cigarette smokers.

Thomas et al. (*25*) investigated the phagocytic function in mice chronically exposed to cigarette smoke, and the effects of *in vitro* exposure to cigarette smoke on macrophage activity. Cultures of radiolabeled *Pseudomonas aeruginosa* were employed to investigate phagocyte activity *in vivo* and *in vitro*. Mice were exposed on weekdays to fresh cigarette smoke for periods up to 37 weeks, and the bactericidal and clearance activity of their lungs were measured. Both pulmonary clearance and bactericidal activity were impaired. They concluded that macrophages exposed to cigarette smoke *in vitro* initially had a depressed phagocytic rate, but if phagocytosis over a prolonged period was measured, it was eventually enhanced over the rate of control macrophages, and the vapor phase of cigarette smoke could also transiently inhibit and then enhance the phagocytic activity. Huber et al. (*26*) suggested that chronic exposure to tobacco smoke does not impair, and in fact may stimulate, the host defenses of the lung, as evaluated by *in vivo* and *in vitro* pulmonary AM function.

In contrast, Drath et al. (*27*) found that 30 days of exposure to tobacco smoke caused an initial enhancement of phagocytic activity which was later reversed with more chronic exposure, and finally, there was a definite inhibition of uptake. This may indicate that the effect of smoke on phagocytosis is cumulative.

In an *in vitro* phagocytic system using rabbit pulmonary AM, small amounts of cigarette smoke quantitatively inhibited the capacity of the cells to inactivate *Staphylococcus albus* bacteria. It is suggested that the role of cigarette smoke in the pathogenesis of pulmonary diseases may be mediated through an inhibition of the phagocytic activity of AM (*28*).

The increase in secretion of lysozyme in macrophages from both humans and rodents exposed to smoke has been reported. This increase is associated with the production of new lysosomes and not just an increase in enzyme content within the original lysosomes (*29*). Macrophages exposed to cigarette smoke also contain less succinate dehydrogenase, cytochrome oxidase, acid phosphatase, NADH diaphorase, and nonspecific esterase and have an impaired ability to synthesize protein (*30*). Not only do smokers have a greater number of macrophages, but the cells also produce greater quantities of elastase (*31*). The secretion of such enzymes may lead to subsequent tissue damage and connective tissue degradation (*32*). Human macrophages from smokers also release increased amounts of O_2 (superoxide anion). Such stimulation may be a significant factor in the pathogenesis of emphysema. Cigarette smoke produces approximately a twofold increase in oxygen consumption and hydrogen peroxide release in response to phagocytosis. Studies have also shown that macrophages from smokers have a significantly higher aryl hydrocarbon hydroxylase activity than similar cells from nonsmokers (*33,34*). This increased enzyme activity is important, since this enzyme system can metabolize polycyclic aromatic hydrocarbons to more active carcinogens.

Effects of Tobacco and Related Substances on Metabolic Events in the Lung

Uptake and Metabolism of Nicotine by the Lung

Tobacco smokers commonly inhale smoke into their lungs, from which some of the smoke components transfer to the blood. Although components such as nicotine transfer from lung tissue to the circulation extremely rapidly, it is important to elucidate whether the lung plays an active part in the fate and subsequent distri-

bution of these components. The lung is the first organ in the body to come into contact with nicotine inhaled in tobacco smoke, and thus the possibility exists that a first-pass pulmonary metabolism of nicotine could be occurring which would be analogous to the first-pass liver metabolism of orally administered drugs such as lidocaine (*35*) and aspirin (*36*) in humans. If such a phenomenon did occur, the effective dose of nicotine reaching the systemic circulation could be lowered, but then the metabolites of nicotine would be expected to reach the systemic system and could cause adverse health effect. Hoffman et al. (*37*) recently presented data that clearly illustrate that from nicotine a number of nitrosamines can be found including *N'*-nitrosonornicotine/nicotine-derived nitrosaminoketone, which are strong carcinogens.

In order to investigate the possibility of pulmonary first-pass metabolism of nicotine inhaled in tobacco smoke, McGovern et al. (*38*) studied the absorption and disposition of ^{14}C-nicotine in an isolated perfused rabbit lung preparation after nicotine administration directly into the perfusing blood and tobacco smoke administration via the inspired tracheal air. After administration into the perfusing medium, the rate of nicotine metabolism was first-order and dose-independent at the two doses studied (0.1 and 1.0 mg), but lung metabolic clearance was quite low (3 mL/min) relative to whole body clearance (140 mL/min), measured by administering ^{14}C- nicotine to intact rabbits. Accumulation of nicotine in the lung was 13 to 23% of the dose administered. After administration of tobacco smoke from ^{14}C-nicotine-spiked cigarettes, absorption of nicotine was rapid, but the rate of metabolism was markedly reduced compared with the studies in which the drug was administered in the perfusing medium. This reduction in the rate of metabolism was apparently caused by some component of tobacco smoke; McGovern et al. concluded that the slow clearance of nicotine by the rabbit lung, which was further reduced after smoke administration despite a high pulmonary blood flow rate made the possibility of significant first-pass lung metabolism in smokers unlikely.

Turner et al. (*39*) investigated metabolism of nicotine by the isolated perfused dog lung. ^{14}C-nicotine (50 μg every 30 sec for 10 min administered via the pulmonary artery) undergoes first-pass metabolism to a small extent. ^{14}C-cotinine was detected in the venous blood. Of the injected activity, 6% was in the lung at the end of experiment, 60% being present as ^{14}C-nicotine, and 20% as ^{14}C-nicotine-1'-oxide. But when ^{14}C-nicotine was administered in cigarette smoke, a greater degree of metabolism was observed at first-pass. Pyrolysis products of ^{14}C- nicotine also were present in the venous blood. Lungs after smoke exposure contained 30% of administered radioactivity, with a substantial proportion of ^{14}C-nicotine-1'-oxide. Administration of ^{14}C-nicotine-labeled smoke-to-lung preparations, on closed circuit, gave significant amounts of ^{14}C-cotinine and other metabolites over a 2 hr period. Lung tissue contained approximately 40% of injected dose, of which 25% was ^{14}C-nicotine. Large proportions of ^{14}C-cotinine and ^{14}C-nicotine-1'-oxide were present, but 45% of the activity was present as other unidentified pyrolysis products. Turner et al. (*39*) demonstrated the usefulness of the isolated lung perfusion technique as a tool for the study of the metabolism of smoke constituents and illustrated the importance of inhalation administration when assessing the capability of lung tissue to metabolize drugs whose normal routes of entry into the organism are via the respiratory tract.

Effects of Cigarette Smoke on the Metabolism of Benzo[α]pyrene by the Lung

Smoking is an important risk factor in lung cancer, which is most often bronchogenic. Cigarette smoke contains many carcinogenic compounds such as benzo[α]pyrene, which have to be metabolically activated in order to exert their carcinogenic effects (*1*). Benzo[α]pyrene is metabolized by aryl hydrocarbon hydroxylase to epoxides, which can be converted to phenols, dihydrodiols, glutathione, glucuronide, or sulfate conjugates. It has been suggested that the harmful effects of benzo[α]pyrene are due to covalent interactions of electrophilic metabolic intermediates, e.g., epoxides and diolepoxides, with proteins and nucleic acids (*40,41*). Cigarette smoke increases the activity of aryl hydrocarbon hydroxylase in rat lung (*42*) and in human alveolar macrophages (*43*). Okamoto et al. (*44*) demonstrated that the aryl hydrocarbon hydroxylase activity in hamster lung instilled intratracheally with tobacco smoke condensate, marijuana smoke condensate, and benzo[α]pyrene was significantly higher than that observed in the control. The amounts of many metabolites of benzo[α]pyrene formed by rat lung microsomes (*45*) and by isolated perfused rat lungs (*46*) are increased after cigarette smoke exposure. Simberg et al. (*47*) investigated the stimulatory effect of cigarette smoke on the metabolism and covalent binding of benzo[α]pyrene in the trachea of the rat. The activity of aryl hydrocarbon hydroxylase was increased in the trachea of rats exposed to cigarette smoke for 1 hr daily for either 1 or 10 days. However, the degree of increase in activity was lower in the trachea than in the lung. After a single exposure, the activity in the trachea was at its highest level 12 hr following exposure, but had returned to the control level within 24 hr. The amounts of covalently bound metabolites of benzo[α]pyrene were increased 2-fold in nucleic acid and protein fractions of the trachea when the rats were sacrificed 12 hr after a single cigarette smoke exposure. Simberg et al. concluded that no change could be detected in the activity of epoxide hydratase in the trachea and that repeated cigarette smoke exposures slightly increased the activity of UDP-glucuronosyltransferase both in the trachea and in the lung.

Effect of Cigarette Smoke on Angiotension I Conversion in the Lung

Conversion of the inactive decapeptide, angiotensin I, to the octapeptide pressor agent, angiotensin II, occurs primarily in the lung (*48,49*). There have been several reports of altered pulmonary conversion of angiotension I by changes in pulmonary oxygenation (*50*) and hemodynamics (*51*), and altered plasma coverting enzyme activity has been reported in patients with spontaneous pneumothorax (*52*), sarcoidosis (*53*), and other chronic lung diseases (*54*). In view of the observation that, in spite of the acute pressor effects of smoking, smokers in general tend toward slight hypotension with respect to nonsmokers (*55*), it might be interesting to examine the possible effect of cigarette smoke inhalation on the conversion of angiotensin I to angiotensin II in the isolated perfused lung.

Hagedron et al. (*56*) investigated the effect of tobacco smoke on the biotransformation of angiotensin I in the isolated perfused rabbit lung. Lungs were perfused with Krebs-Ringer bicarbonate buffer in a nonrecirculation fashion at a flow of 20 or 28 mL/min. The lung preparation was permitted to stabilize for 15 to 30 min prior to angiotensin I administration. Angiotensin I was administered at a rate of 81 ng/min in a volume of 0.27 mL/min, via a cannula placed in the perfusion fluid flow in the pulmonary artery. One mL perfusate samples were collected in polypropylene tubes at the outflow from the left atrial cannula at convenient intervals following initiation of the angiotensin I infusion. Smoke was administered to the lung preparation 15 min after initiation of the angiotensin I infusion. Puffs of 2 sec duration and 40 mL volume were generated once per minute from University of Kentucky 2RI Research Cigarettes. Four such puffs were delivered to the lung in each smoke exposure. Angiotensin II concentrations in perfusate fractions were determined by radioimmunoassay, using angiotensin II antiserum and angiotensin II tracer. Under the conditions of these experiments, the extent of angiotensin I conversion to angiotensin II was between 37 and 63% for various lung preparations, and concentration of angiotensin II in perfusate at steady state before and after smoke administration did not show any significant change. It is therefore concluded that the acute administration of cigarette smoke does not significantly alter pulmonary conversion of angiotensin I to angiotensin II.

Bakhle et al. (*57*) investigated the effects of cigarette smoke on the metabolism of vasoactive hormones in isolated lungs of male adult Wistar rats. Rats were exposed to cigarette smoke in an inhalation chamber. During 1 hr, the rats inhaled the smoke from five commercial cigarettes, each of them containing 1 mg nicotine and 16 mg tar, as quoted by the manufacturer. The rats were exposed once (1 day exposure) or daily for 10 consecutive days (10 days of exposure). About 20 hr after the last exposure, rats were anesthetized with sodium pentobarbitone (50 mg/kg, IP), and the lungs were removed and prepared for perfusion. Oxygenated, warmed Krebs solution was pumped through the pulmonary circulation at 8 mL/min. The effluent superfused two assay tissues (rat colon) arranged in cascade below the lung. The contractions of the tissues were recorded on a potentiometric recorder via Harvard smooth muscle transducers and auxotonic levers. Percent conversion of angiotensin I to angiotensin II was calculated from these records, and they concluded that angiotensin I conversion was increased after 1 day exposure but after 10 days exposure conversion returned to normal.

Tivonen (*58*) investigated the effect of nicotine instead of cigarette smoke on conversion of angiotensin I in isolated lungs of male adult Wistar rats. Rats were exposed to SC injections of nicotine base (1 mg/kg). When the rats were exposed to nicotine only once and 50 ng of angiotensin I was injected as a bolus into the isolated perfused lungs from these animals about 24 hr after the nicotine exposure, there was no difference in the activation of angiotensin I to angiotensin II when compared with the lungs from sham treated or control animals. When the nicotine exposure was repeated during 10 consecutive days, there was an increased formation of angiotensin II from angiotensin I when compared with control rats. There was, however, no difference between the lungs from 10 days sham and 10 days nicotine-exposed rats. This suggests that the increased formation of angiotensin II might have been due at least partly to factors other than nicotine, for instance, to the activation of renin-angiotensin system because of the stressing of the animals. These results differ from those of Balkhle et al (*57*). The discrepancy in the results of these two studies suggests that nicotine was not the factor in cigarette smoke which caused the metabolic changes in the cigarette smoke exposure experiments. However, it must be taken into account that in the present work nicotine reached the lungs via circulation, thus affecting first the endothelial cells responsible for angiotensin II conversion (*59*); much lower amounts of IV nicotine caused endothelial injury (*60*). When cigarette smoke is inhaled, nicotine is partly transferred through the alveolar wall and it could thus have better access to the enzymes metabolizing prostaglandin E_2. The activation of angiotensin I conversion after cigarette smoke inhalation might also be secondary to the changed prostaglandin metabolism. Ercan et al. (*61*) have proposed that when one of the enzymes, PG-synthetase or converting enzyme, is inhibited, the other is simultaneously activated.

As previously described (*6*), we also investigated the effects of cigarette smoking on serum angiotensin I converting enzyme (ACE) activity in healthy volunteers. Figure 10 shows the effect of cigarette smoking on serum ACE level. Serum ACE activity showed a significant increase immediately after smoking and returned to the control level 20 min after smoking. In this experiment we did not measure conversion of angiotensin I to angiotensin II. If the increase of ACE activity means increase of conversion of angiotensin I to angi-

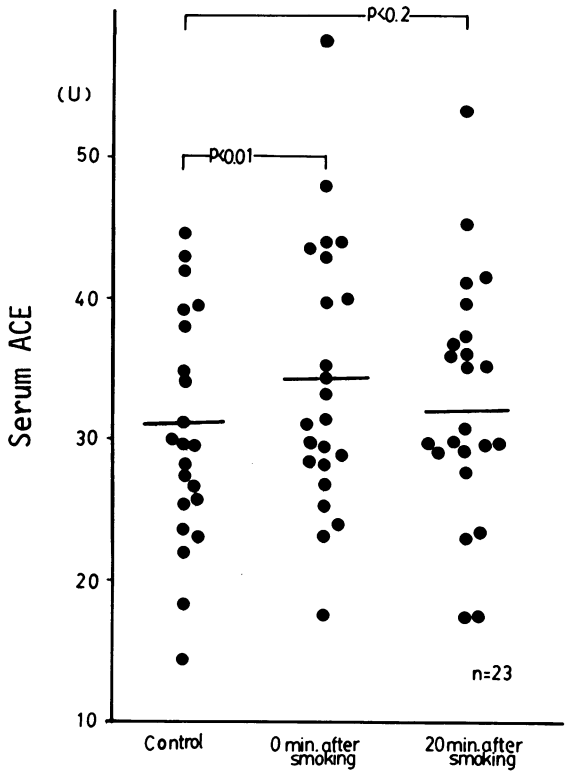

FIGURE 10. Effect of cigarette smoking on serum level of ACE.

otensin II, the events at the pulmonary endothelial cell membrane could be affected by a relatively distant stimula, i.e., smoke in the airways.

Effect of Cigarette Smoking on Serotonin (5-HT) Metabolism in the Lung

Pre-exposure of rats to cigarette smoke for 1 hr either once or daily during 10 consecutive days did not affect the metabolism of 5-hydroxytryptamine (5-HT) in isolated perfused lung (*62*). However, there is evidence that cigarette smoke may change 5-HT metabolism. Perfusion of isolated lungs of the rat (*63*) and guinea pig (*64*) with anoxic solution partially inhibits both the 5-HT uptake and the oxidation of 5-HT. Cyanide, a component of cigarette smoke, also inhibits the uptake of 5-HT (*64*). In mouse skin, cigarette smoke exposure inhibits 5-HT-specific monoamine oxidase activity and increases 5-HT uptake (*65*).

Karhi et al. (*66*) investigated whether cigarette smoke ventilation during perfusion of isolated rat lungs has any effect on the pulmonary metabolism of 5-HT. ^{14}C-5-HT was infused into the pulmonary circulation of isolated perfused rat lungs. The nonrecirculating perfusion effluent was collected during the 5-HT infusion in three consecutive 1-min fractions. The amount of metabolites of 5-HT was determined from the perfusion effluent and from the perfused lungs. The amount of metabolites of 5-HT in the perfusion effluent was decreased during cigarette smoke ventilation, and the amount of metabolites of 5-HT in the perfused lungs was also decreased by cigarette smoke ventilation, although the total amount of radioactivity in the lung tissue was not significantly changed. The decreased pulmonary inactivation of 5-HT may cause increased circulation levels of 5-HT, which would explain some cardiovascular changes during smoking.

As previously described (*6*), we also investigated the effect of cigarette smoking on plasma level of serotonin in 23 healthy volunteers (20 men and 3 women). All of the volunteers were heavy smokers (20 to 40 cigarettes/day) for 5 to 20 years. The volunteers stopped smoking at least 12 hr before the testing began. When testing began, men smoked five cigarettes and women smoked three cigarettes within 10 min. Figure shows changes of plasma serotonin level in 23 healthy volunteers after cigarette smoking. Plasma serotonin level showed a significant decrease immediately after smoking and stayed lower than normal for more than 20 min. The discrepancy in the results of this study and the one by Karhi et al. might come from the difference of animal species and from the difference of experiment method, i.e., *in vitro* or *in vivo*.

Although the lung is thought to be the major site of serotonin inactivation, blood platelets, liver, and other tissues can also bind or inactivate this amine. Further studies are needed in this field before the role of altered serotonin metabolism in the course of cigarette smoke-induced cardiovascular alterations can be evaluated.

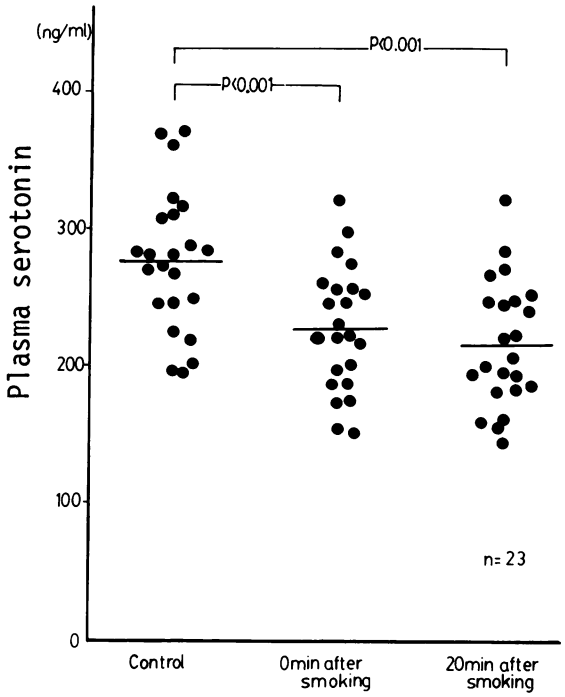

FIGURE 11. Effect of cigarette smoking on plasma level of serotonin.

Effect of Cigarette Smoking on Histamine Metabolism in the Lung

Histamine was not removed by the pulmonary circulation in anesthetized dogs, as the biological activity of blood sampled from the femoral artery was the same whether histamine was infused into the superior vena cava or into the base of the ascending aorta or left ventricle (*67*). Although histamine was not removed in the pulmonary circulation in whole lungs, it was readily inactivated in lung slices or in chopped or homogenized lung preparations from man (*68*), cat (*69*), rabbit (*70*), guinea pig, and rat (*71*). The main metabolite formed was species dependent and reflected the types of histamine-metabolizing enzymes in the lung of each species (*72*) and their concentrations in the lung. The lack of histamine inactivation in whole lung might be due to the absence of a transport mechanism for histamine in the pulmonary circulation. The lung has the capacity to synthesize histamine, as histidine decarboxylase is present in lung tissue (*73*). Histamine is present in the lungs of most species; the amount is generally related to the population of mast cells. These cells can take up and store histamine, but uptake is a slow process and not likely to contribute to the removal of exogenous histamine during a single passage through the pulmonary circulation.

The release of histamine from human asthmatic lung and bronchial tissue during challenge with specific antigen was demonstrated by Schild et al. (*74*) and confirmed by Brocklehurst (*75*), who perfused and challenged lung segments from subjects with allergies and showed the release of both histamine and slow-reacting substance of anaphylaxis (SRS-A).

The release of histamine has been postulated to occur in two experimental situations. First, during anoxemia in a heart-lung preparation, the increase in force of myocardial contraction can be blocked by the previous administration of the antihistaminic drug (*76*). Second, the bronchoconstrictor response in the intact dog following the inhalation of tobacco smoke, the bronchial arterial injection of nicotine, or the pleural application of nicotine solution can be simulated by the corresponding administration of histamine, so the release of histamine was proposed as the basic mechanism for the bronchoconstriction induced by tobacco and nicotine. Aviado et al. (*60*) investigated the release of histamine during inhalation of cigarette smoke and anoxemia in the heart-lung and intact dog preparation. They suggested that there was a definite release of histamine during the period of anoxia, and that the initial bronchoconstriction initiated by inhalation of cigarette smoke might induce a localized state of anoxia, and this in turn might cause the release of histamine.

As previously described (*6*), we investigated the effect of cigarette smoking on plasma level of histamine in healthy volunteers. Figure 12 shows the change of plasma histamine level in 23 healthy volunteers by cigarette smoking. Plasma histamine level showed a significant decrease immediately after smoking and stayed lower than normal for more than 20 min. These results suggest that plasma histamine levels were markedly decreased after smoking by either the elevation of histaminase activity or increase of histamine uptake induced by nicotine and other components of tobacco smoke.

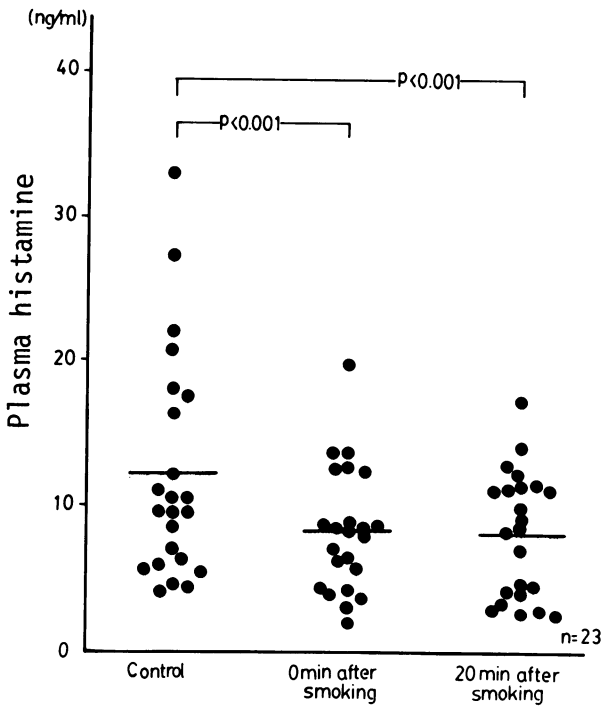

FIGURE 12. Change of plasma histamine level before (control), immediately after (0 min), and 20 min after cigarette smoking.

Effect of Cigarette Smoke and Related Substances on Prostaglandin and Thromboxane Metabolism in the Lung

Any contemporary account of metabolic functions of the lung might be incomplete without a discussion of prostaglandin (PG) metabolism. The prostaglandins possess a bewildering spectrum of physiological and biochemical activities in the various mammalian tissues and organs, including heart and lung. It is well known that the lung possesses extremely efficient enzymatic machinery for the rapid conversion of prostaglandins to inactive metabolites, and that the lung also has an ability to synthesize prostaglandins. This is surprising considering that the lung contains a relative abundance of PG synthetase and considering that prostaglandins are readily released from perfused lungs by such diverse stimuli as vasoactive peptides (*78*), phospholipase A (*79*), anaphylactic shock (*80*), histamine (*81*), serotonin and tryptamine (*82*), hypoxia (*83*), and mechanical ventilation (*84*).

Prostacyclin is a product of arachidonic acid metab-

olism generated by the vessel wall of all mammalian species, including man. Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation. It inhibits platelet aggregation through stimulation of adenylate cyclase, which leads to an increase in cyclic AMP in the platelets. The enzyme which synthesizes prostacyclin is mainly located in the endothelial layer of the vascular wall (*85*).

In blood platelets, arachidonic acid is converted by the enzyme thromboxane synthetase to a potent vasoconstrictor and proaggregation substance, thromboxane A_2 (*86*). Therefore, arachidonic acid is metabolized in the vessel wall and in platelets to potent substances with opposing biological activities. The balance between thromboxane A_2 and prostacyclin might be important in the control of the pulmonary circulation.

Because blood platelets appear to play a central role in the initiation of arterial thrombosis, the difference in aggregation behavior in platelets from smokers and nonsmokers seems to be important. It is known that smoking induces enhancement of platelet function (*87*). Evidence supporting this idea includes an association of smoking with increased ADP-induced platelet aggregation (*88*), a shortening of platelet survival (*89*), and increased thromboxane synthesis (*90*). In addition, there is some evidence that smoking might exert its action by reducing vascular prostacyclin (PGI_2) synthesis (*91*). Burghuber et al. (*92*) investigated the response of platelets to exogenous PGI_2 in chronic smokers and nonsmokers before and after smoking two cigarettes and concluded that decreased platelet sensitivity to PGI_2 might be an important contributing factor to the altered platelet function observed in patients with atherosclerosis.

Hagedron et al. (*93*) studied the effect of tobacco smoke on uptake and metabolism of prostaglandin $F_{2\alpha}$ in isolated perfused rabbit lungs. Male white rabbits weighing 3 to 4 kg were anesthetized with sodium pentobarbital at a dose of 40 mg/kg and were treated with heparin at a level of 1000 U/kg. Blood was withdrawn by cardiac puncture; the lungs and heart were removed; and the pulmonary artery, left atrium, and trachea were cannulated. The lung preparation was then suspended in an artificial thorax. Respirator controls were set to provide 50 respirations per min, with 30 mL stroke volume. Perfusate flow was at a constant hydrostatic pressure of 23 cm, and flow prior to $PGF_{2\alpha}$ administration was stable at 200 to 300 mL/min. $PGF_{2\alpha}$ was adminstered to the lung both as a bolus dose and as a constant rate infusion. In the bolus dose studies, $PGF_{2\alpha}[9\text{-}^3H(N)]$ was combined with unlabeled $PGF_{2\alpha}$ and was administered as a bolus dose of 0.28 to 0.42 μmole (15-30° μCi) in 0.25 to 0.4 mL of normal saline. In the constant infusion studies, $PGF_{2\alpha}$ was administered to the lung via an infusion pump which delivered 0.17 nmole $PGF_{2\alpha}$(1.59 μCi)/min in an infusion fluid volume of 0.53 mL/min. In these studies, smoke was administered to the lung after 30 min from starting perfusion without altering the constant rate of $PGF_{2\alpha}$ infusion. In smoke exposure studies, puffs of 2-sec duration and 40 mL volume were generated once per minute from University of Kentucky 2RI Research Cigarettes. Four such puffs were delivered to the lung in each smoke exposure. These studies showed that there was no effect of cigarette smoke on uptake and metabolism of $PGF_{2\alpha}$ in isolated perfused rabbit lungs.

Bakhle et al. (*57*) studied the effects of cigarette smoke on the metabolism of vasoactive hormones in isolated rat lungs. They demonstrated that inactivation of PGE_2 was decreased after 1 day exposure and after 10 days exposure, there was a further decrease that could not be attributed to smoke alone. PGE_2 metabolism in rat lung is not transport limited, and this metabolism was unequivocally decreased after 1 day exposure to cigarette smoke, suggesting a decreased activity of 15-hydroxyprostaglandin dehydrogenase (PGDH), the rate- limiting enzyme in PGE_2 degradation in lung. Although the change is numerically small, it represents a doubling of the PGE_2 entering the systemic arterial circulation. The changes occurring after 10 days exposure can not be safely attributed to smoke exposure, as survival of PGE_2 increased in both sham- and smoke-exposed animals.

Tivonen (*58*) studied the effect of nicotine on the metabolism of PGE_2 in isolated rat lungs. Usually, about 95% of PGE_2 (250–500 ng) was inactivated during one passage through the pulmonary circulation, but exposure of rats to nicotine for 1 or 10 days did not change the ability of their lungs to inactivate PGE_2. The discrepancy in the results of these two studies suggests that nicotine was not the factor in cigarette smoke which caused the metabolic changes in the experiments. When cigarette smoke is inhaled, nicotine is partly transferred through the alveolar wall, and it could have better access to the enzymes metabolizing PGE_2. Berry et al. (*94*) also demonstrated that nicotine aerosol inhalation increased prostaglandin efflux from isolated rat lungs, and in their preliminary experiments, $PGF_{2\alpha}$ breakdown was inhibited in rat lung homogenates by nicotine.

Effect of Cigarette Smoke on Protein Synthesis in the Lung

Garrett et al. (*95*) studied the effect of cigarette smoke on protein synthesis in the lungs of Sprague-Dawley rats. *In vivo* exposure of lung to cigarette smoke results in suppression of protein synthesis by pulmonary tissue. Lungs from sham control rats synthesize protein at a rate of 0.97 ± 0.16 nmole proline/mL DNA/min. Pulmonary protein synthesis during exposure to 45 to 60 puffs of smoke is inhibited by 60 to 80%. The component of smoke which inhibits protein synthesis is not significantly removed by Cambridge filters which adsorb particulate matter or by charcoal filters which adsorb many gases. Carbon monoxide is not removed by either type of filter. At the level present in cigarette smoke, CO produces a marked protein inhibitory effect comparable to that of cigarette smoke (*96*). These results suggest that CO might be a component in cigarette smoke that interferes with pulmo-

nary protein synthesis. Inhibition of protein synthesis in the lung of intact animals might result in defective structural composition and could render the lung susceptible to injuries inflicted by bacteria or other environmental agents. Garret et al. suggested that emphysema might be due to proteolysis of the lung at a time when it was unable to repair itself adequately through protein synthesis.

Effect of Cigarette Smoke Exposure on Testosterone Metabolism in the Lung

Hartiala et al. (*97*) investigated the effects of cigarette smoke exposure on testosterone metabolism in the isolated perfused rat lung. They exposed rats to cigarette smoke daily for 1 hr for 5 consecutive days. The metabolism of [4-^{14}C]-testosterone by isolated perfused lungs was studied on the sixth day. Slightly diluted rat blood, containing the radioactive substrate, was used as the perfusion fluid. Compared to sham-exposure rats, the formation of reduced metabolites, dihydroxysteroids, was less, and the metabolism of testosterone diminished after cigarette smoke exposure. After the perfusion, the amount of polar nonconjugated metabolism was significantly higher in the smoke-exposed lung tissue than in the control lungs, and no conjugate formation was observed. These researchers suggested that the decrease in the formation of reduced metabolites was due to an inhibition of the 4-ene-5α-reductase activity. The present study clearly calls for further investigations on the effects of cigarette smoking on the steroid metabolism.

Effect of Cigarette Smoke Exposure on Cyclic Nucleotides Metabolism in the Lung

Nitroglycerin, nitroprusside, nitrosamines, sodium azide, nitric oxide, peroxides, and the hydroxyl radical activate guanylate cyclase to convert guanosine triphosphate into cyclic GMP. Although the mechanism of enzyme activation is not clear, it is apparent that biological oxidation and radical formation are very important factors in the function of guanylate cyclase (*98*). Based upon this information and the fact that nitric oxide is an important gaseous constituent of tobacco smoke, Arnold et al. (*99*) exposed lung tissue *in vitro* and guanylate cyclase preparations from lung tissue to an environment of pure (98%) cigarette smoke, and showed prompt increases in cyclase activity and cyclic GMP levels. In a variety of mammalian systems, cyclic GMP has been proposed to act as a mediator of tissue proliferation (*100*), smooth muscle contraction and relaxation (*101*), and as a promoter of the inflammatory response (*102*). Because many of these actions have been applied to tissue cyclic GMP, it seems possible that the activation of guanylate cyclase and production of cyclic GMP might be an important pulmonary response to cigarette smoke.

Cigarette smoke is an extremely complex mixture of particulate and gaseous materials. Veseley et al. (*103*) have shown that hydrazine, a known carcinogen that is a component of cigarette smoke, is capable of stimulating guanylate cyclase *in vitro*. Klass (*104*) studied whether exposure of rats *in vivo* to tolerable levels of 3cigarette smoke is capable of altering lung cyclic GMP. Adults rats were anesthetized with pentobarbital, and ventilation with mixtures of air and cigarette smoke at 10 cm H_2O inspiratory pressure was achieved after a tracheotomy was performed. Lung tissue samples were taken at intervals during the 20-min expsoure period and analyzed for levels of cyclic adenosine 3′, 5′-monophoshate (AMP), and cyclic GMP. Blood carboxyhemoglobin (COHb) levels at 5 min and 15 min of exposure were high, but levels of COHb were sublethal. Lung tissue cyclic AMP was unchanged with this exposure, but cyclic GMP levels rose dramatically. Cyclic GMP has been suggested to play a role in the action of bronchial and tracheal smooth muscle (*105*), though the exact nature of this mechanism is unclear. It is possible to speculate that an irritative response to cigarette smoke triggers bronchial smooth muscle contraction with an associated increase of cyclic GMP. Cigarette smoke has also been shown to induce pulmonary vascular constriction which may be associated with increased level of cyclic GMP. A similar response might equally accompany bronchial gland secretion stimulated by cigarette smoke (*106*). Barnett et al. (*107*) have demonstrated increased levels of cyclic GMP in the dog lung tissue by histamine infusion, and Kaliner et al. (*102*) have demonstrated cyclic GMP to be associated with increased chemical mediator release from lung. It is possible that the increase in lung cyclic GMP seen here is related to inflammation associated with cigarette smoke inhalation. Since changes in cyclic GMP metabolism have been associated with abnormalities in cellular growth (*100,103*), it is possible that the increase in cyclic GMP in tissues associated with components of cigarette smoke might be related to the initiation of abnormal cellular growth such as bronchogenic carcinoma.

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